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The canonical and accessory Sec system of Bacteria

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Current Topic in Microbiology and Immunology. **2016**

CHAPTER ONE

The canonical and accessory Sec system of Bacteria

Abstract – The Sec system is present in all bacteria and responsible for the translocation of the majority of proteins across the cytoplasmic membrane. The system consists of two principal components: the ATPase motor protein, SecA, and the protein-conducting channel, SecYEG. In addition to this canonical Sec system, several Gram-positive bacteria also possess a so-called accessory Sec system. This is a specialized translocation system that is responsible for the export of a subset of secretory proteins, including virulence factors. The accessory Sec system consists of a second SecA paralog, termed SecA2, with or without a second SecY paralog, termed SecY2. In some bacteria, the accessory Sec system is dependent on the canonical Sec system for functionality, while in other bacteria, they can function independently. In this review, we provide an overview of the current knowledge of the canonical and accessory Sec system of bacteria with a focus on the primary component of the Sec translocase, SecA and SecYEG.

1. Introduction

Bacteria are favorite model organisms in scientific research to study cellular processes that also occur in multicellular organisms. Cells are equipped with mechanisms to transport proteins from the site of synthesis, i.e., the cytoplasm, to the cell envelope or to the extracellular environment. This process is not only important for the survival of the cell, but also plays important roles in pathogenesis, and for instance the interaction with eukaryotic hosts. The major route for protein transport in bacteria is provided by the general secretion pathway (Sec pathway). The Sec pathway and its functional components has been extensively studied in the Gram-negative bacterium *Escherichia coli*, and also in Gram-positive bacterium, *Bacillus subtilis* [1–5]. Protein transport in this pathway is mediated by the Sec translocase, which in its minimal form consists of a protein-conducting channel formed by the heterotrimeric membrane protein complex, SecYEG, and the essential ATPase SecA that acts as a molecular motor [6].

In general, the Sec system of Gram-negative and Gram–positive bacteria is similar in composition, and main components are highly conserved. In the last decade, however, studies on the Gram-positive Sec system revealed some interesting differences. Besides the canonical Sec components, a large number of Gram-positive bacteria possess accessory Sec components that are not found in Gram-negative bacteria. This concerns a presence of a second SecA paralog, termed SecA2, either with or without a second SecY paralog, termed SecY2 [7–9]. In contrast to the canonical Sec translocase, which is essential for the translocation of the majority of secretory proteins, the accessory SecA2 and SecY2, in most cases, appear not essential [7–9]. They seem to be especially important for the export of a subset of proteins, which in some bacteria are mostly virulence factors [10–12]. In bacterial species that possess both SecA2 and SecY2, e.g., *Streptococcus*, the accessory components form a separate translocation system to export specific substrates independently of the canonical Sec system [13]. Interestingly, in species that possess only SecA2, e.g., *Mycobacteria*, SecA2 seem to work together in conjuction with the canonical SecYEG/SecA1 translocase in the export of multiple substrates [13–16]. Here, we discuss the current knowledge on the canonical and accessory Sec system in bacteria. We will mainly focus on the primary component of the system, the motor protein SecA and the protein membrane channel SecY, and highlight the potential mechanistic implications.

1.1. Sec pathway, the major route for protein secretion

The Sec pathway forms a conserved route for the secretion of housekeeping proteins, and for protein secretion in general. Secretory proteins are initially synthesized at the ribosome as unfolded preproteins with a cleavable N-terminal signal peptide. The signal peptide is important to distinguish preproteins from cytoplasmic proteins, adds to the unfolding of the associated mature domain, and targets these proteins to the translocation sites at the membrane [17,18]. Targeting is mostly a post-translationally event, but some secretory proteins are targeted co-translationally [19].

During post-translational targeting, protein synthesis is first completed at the ribosome before the preprotein engages with the Sec system at the membrane. The molecular chaperone stabilizes the preproteins in a translocation-competent state and directs them to the translocation site. At the membrane, the molecular chaperone transfers the preprotein to the SecA motor domain of the Sec translocase. In the next steps, multiple cycles of ATP binding and hydrolysis by SecA result in the stepwise translocation of the unfolded preprotein through the SecYEG channel [20–22]. Another heterotrimeric membrane protein complex, SecDFyajC, stimulates translocation and utilizes the proton motive force (PMF) to facilitate this process [23,24]. Generally, the post-translocation mechanism is similar in Gram-negative and Gram-positive bacteria. One main difference is in the nature of the molecular chaperones. In Gram-negative bacteria, the chaperone function is carried out by SecB [25] or other general chaperones like Trigger factor, DnaK or GroEL [26]. In Gram-positive bacteria that lack a SecB homolog, the chaperone CsaA has been implicated in protein translocation [27,28]. It should be noted that the more general chaperones do not entail the specific targeting function of SecB.

During co-translational targeting, preproteins remain bound to the ribosome as a nascent chain, and are targeted to the SecYEG channel by signal recognition particle (SRP) and the signal recognition particle receptor, FtsY. SRP and FtsY are both GTPases, and release of the nascent chain from SRP to the SecYEG channel is facilitated by heterodimerization of SRP and FtsY and subsequent GTP hydrolysis. Mostly, nascent membrane proteins utilize the aforementioned targeting route for co-translational membrane insertion, albeit there is a subset of secretory proteins that use this pathway as well. However, preprotein translocation is strictly dependent on the ATPase SecA and ATP hydrolysis. After translocation, the signal peptide is cleaved from the preprotein by signal peptidase, and the protein will fold on the trans-side of the membrane into its functional conformation, or continue its

passage guided by chaperones to the outer membrane. In Gram-positive bacteria, some proteins interact with the cell wall, while others pass through the cell wall and are released into the external environment. A schematic representation of the general sec pathway in bacteria is shown in Fig. 1.

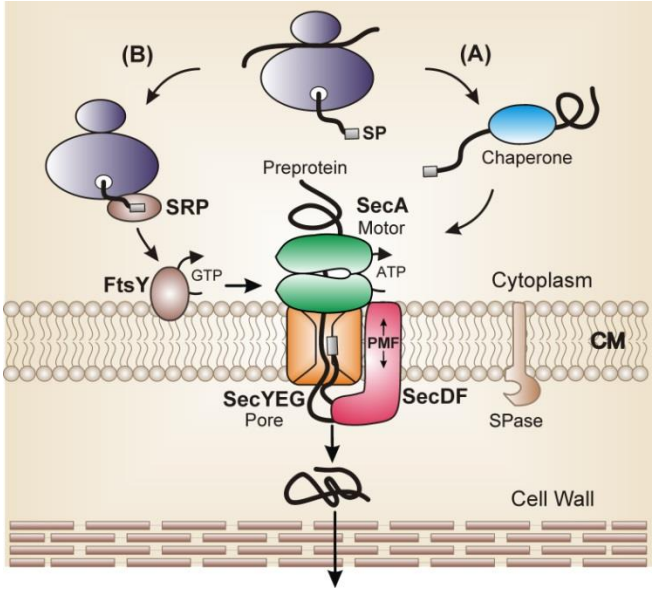

Figure 1. The Sec pathway. (A) Post-translational targeting: preproteins synthesized at the ribosome (purple) are captured in an unfolded state by a chaperone protein (blue), and targeted to SecA (green) that is bound to the SecYEG channel (orange) at the cytoplasmic membrane (CM). SecA pushes the preprotein through the SecYEG channel in an ATP-dependent manner. In addition, SecDFyajC (red) uses the PMF to pull preproteins into the periplasm. After translocation, the signal peptide (SP) is removed by signal peptidase (SPase) and the mature protein is released. (B) In co-translational targeting, preproteins are targeted to the translocation site as a ribosome-nascent chain complex (RNC) by SRP and FtsY (brown) where protein synthesis will commence involving SecA and ATP.

1.2. SecA, the translocation motor

SecA functions as an ATP-driven molecular motor to facilitate protein translocation across the SecYEG protein-conducting pore. SecA is a highly conserved bacterial protein, but also present in chloroplasts of plant cells, where it is needed for protein translocation into the thylakoid. In the cell, SecA exists in a soluble cytosolic form [29] and membrane bound forms i.e., associated with SecYEG [30] and with phospholipids [31–33]. SecA is a homodimeric protein. X-ray crystallographical studies on SecA proteins from different bacteria revealed a

dimer organization either with antiparallel [34–38] or parallel [39] protomers. The SecA protomer can be divided into several structural domains (Fig. 2). The DEAD-motor domain is part of the central core of SecA, and consists of two subdomains: the nucleotide binding fold 1 (NBF1) and NBF2, also called the intramolecular regulator of ATPase activity 2 domain (IRA2). Both NBFs are homologous to the RecA-like nucleotide binding folds found in DNA/RNA helicases [40]. The interface of the NBF1 and NBF2, which comprise the Walker A and B motifs is the site for ATP binding and hydrolysis [41–43]. In addition to the NBFs, SecA contains two substrate specificity domains: the preprotein-binding domain (PBD) or preprotein cross-linking domain (PPXD) and the C domain. The PPXD has been shown to be involved in binding of preproteins [44–46]. The C domain is located at the C terminus of NBF2 and consists of four subdomains: the helical scaffold domain (HSD), that control the opening and closing of the DEAD motor [34,47]; the helical wing domain (HWD); the intramolecular regulator of ATP hydrolysis (IRA1), which acts as an inhibitor of ATP hydrolysis [48]; the C-terminal linker domain (CTL) which contains a zinc finger which is important for the interaction with the secretion specific chaperone SecB [49–51] and phospholipids [52].

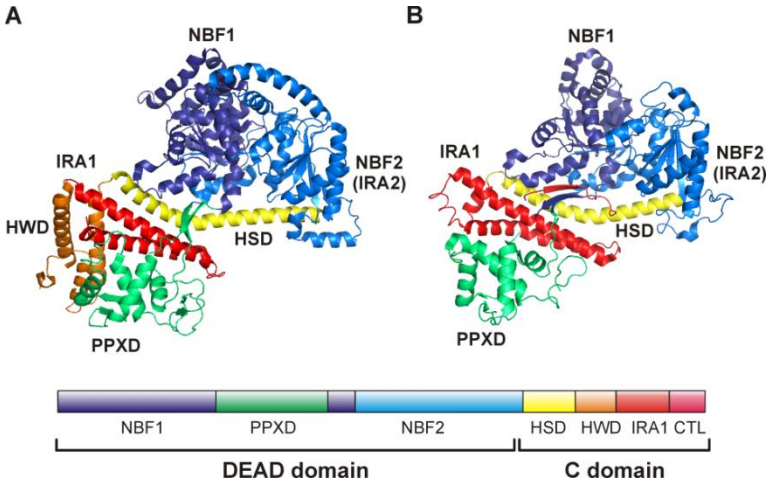

Figure 2. Structure of SecA, the translocation motor. (A) SecA1 protomer from *Mycobacterium tuberculosis* (PDB accession code: 1NKT) and (B) SecA2 from *M. tuberculosis (*PDB accession code: 4UAQ), showing the different subdomains, which are indicated with different color. NBF1 and NBF2, nucleotide-binding folds 1 and 2; PPXD, preprotein cross-linking domain; HSD, α-helical scaffold domain; HWD, α-helical wing domain, IRA1, The intramolecular region of ATP hydrolysis 1; and CTL, C-terminal linker domain.

The oligomeric state of SecA in solution and during protein translocation has been extensively studied with various methods. SecA exists in solution in a dimer–monomer equilibrium with a K_d in nanomolar range (Kusters et al. 2011; Wowor et al. 2011). This suggests that most of SecA must be dimeric in the cell, since the cellular concentration of SecA has been suggested to be in the micromolar range (5–8 μM) [56,57]. The dimer–monomer equilibrium of SecA is shifted towards the monomer at high ionic strength and low temperature [53–55], suggesting that electrostatic and hydrophobic interactions play a critical role in maintaining the dimer. SecA dimerization is also influenced by ligands, such as phospholipids, signal peptides [58–60] and nucleotides [61]. The precise functional state of SecA during protein translocation process is still a matter of debate. A multitude of studies demonstrate that SecA functions as a dimer during protein translocation [54,62–69], although other report suggests that monomeric SecA obtained by extensive mutagenesis retains some activity with a hyperactive SecY mutant [59]. Single molecule studies demonstrate that the dimeric SecA binds the SecYEG translocation pore with high affinity, where one of the protomers binds SecYEG tightly, whereas the other protomer is bound to the SecYEG-bound SecA [54].

1.3. SecYEG, the protein-conducting channel

The Sec translocon shows a heterotrimeric organization that is highly conserved in three kingdoms of life [70]. In bacteria, it consists of three integral membrane proteins SecY, SecE, and SecG [6], which together forms the protein-conducting channel SecYEG complex, that is homologues to Sec61αγβ in eukaryotes and SecYEβ in archaea. The X-ray crystallography structure of *Methanoccoccus jannaschii* SecYEβ provided the first high-resolution insight into the structural organization of the translocation channel [71] (Fig. 3).

The SecY protein forms the actual channel and consists of ten α-helical transmembrane segments (TMSs) that form a clamshell structure, i.e., TMSs 1–5 and TMSs 6–10. The N- and C-termini localize to the cytoplasm. The two TM domains are connected by a periplasmic loop between TMS 5 and TMS 6 forming a clamshell structure. The *E. coli* SecE consists of three TMSs (TMSs 1-3), where the first two TMSs are connected to the third tilted TMS via the amphipathic helix. Only the third TMS and the amphipathic helix is essential for the functionality [72–74]. TMS3 and the amphiphatic helix embrace the SecY clamshell, in which the TMS3 associated with one half of the clamshell, and the amphipathic helix associated with the other half. These two SecE domains are the major sites of SecY-SecE interactions and are important for the stability and flexibility of SecY [75,76]. SecE of Gram-positive bacteria consists only one TMS and the amphipathic helix,

which is homologous to the corresponding functional part of the *E. coli* SecE [77,78]. The Secβ protein, which presumably is homologous to the bacterial SecG is located peripherally in the structure and shows limited contact with SecY. SecG is not essential for the functionality in bacteria, but it increases the efficiency of translocation [79–81], and has also been suggested to associate with SecA [82,83].

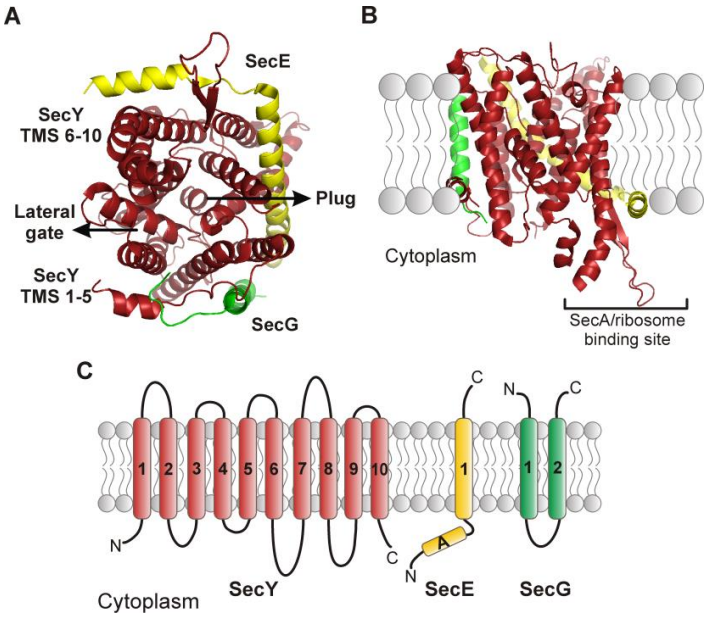

Figure 3. Structure of SecYEG, the protein-conducting channel. (A) Structure of SecYEβ from *Methanoccoccus jannaschii* (PDB accession code: 1RH5), viewed from the cytoplasm. SecY are coloured in red, SecE in yellow, and SecG in green. The lateral gate and the plug are indicated with the arrow line. (B) SecYEβ viewed from the side, in position in the lipid bilayer. (C) Secondary structure prediction of SecYEG from Gram-positive bacteria (*B. subtilis)*. The TMSs are numbered and the essential amphipathic helix of SecE is labeled with A.

The overall structure of SecYEG channel shows an hour glass shape [71]. The central channel is constricted with six isoleucine residues that form a hydrophobic pore ring that acts as a seal to provide a barrier for water and ions [84,85]. Below the pore ring is a small helix called the plug domain. The plug domain together with the pore ring prevent ion flow through the channel in the closed conformation [85]. The structure also contains a lateral gate between TMS 2 and TMS 7 of SecY, which plays an essential role in the lateral insertion of membrane proteins. The signal sequence of preproteins and nascent TMSs were suggested to bind this

lateral gate [86,87], and this initial insertion into the lateral gate is believed to open the channel. The cytoplasmic side of the SecYEG channel contains several binding sites for the cytoplasmic binding partner, i.e., SecA and ribosome [88–90], and is important for protein translocation. SecYEG also interacts with other membrane proteins, such as SecDFyajC [23] and the membrane protein insertase, YidC [91].

2. The accessory Sec translocase

2.1. SecA2, the specialized SecA protein

The first SecA2 protein was identified fifteen years ago in Mycobacteria [7,11]. Nowadays, it clear that SecA2 is present in a large number of Gram-positive bacteria, but absent in Gram-negative bacteria. Interestingly, this accessory SecA2s are not closely related to each other phylogenetically [13]. The sequence similarity between SecA2 and their corresponding SecA homologue (SecA1) varies between bacterial species. In almost all cases, SecA2 proteins are smaller in size, as compared with SecA1s. The first crystal structure of SecA2 was published recently from *Mycobacterium tuberculosis* [92] (Fig. 2). Overall, the structure shows a high similarity to the *M. tuberculosis* SecA1 and other orthologs of the SecA family. Most functional domains are present in the SecA2 structure, including the two NBFs. Despite the similarities, there are some structural differences in SecA2 compared to SecA1. SecA2 is a smaller protein because of several deletions, mostly in the C domain. The main structural difference is the absence of HWD domain in SecA2. The functional significant of the absence of the HWD in SecA2 is unclear, but possibly this hints at a reduced interaction with SecYEG. In SecA, the HWD is important for the interaction with SecYEG [88,93]. In the structure of SecA2, also the orientation of the PPXD domain and the two-helix finger are different from that of SecA1. Additionally, the conserved tyrosine in the two-helix finger of SecA1, which provides the major contact with the SecA1 substrate [94], is missing in SecA2. Overall, these structural differences may contribute to the special and distinct role of SecA2 in the protein export.

In general, the accessory SecA2 has a more specific role than the canonical SecA, as discussed in detail elsewhere [13,15,16]. In contrast to SecA1, which is essential and involved in transport of the majority of proteins, SecA2 in most cases is not essential, and has a specialized function for the export of a subset of proteins. Additionally, SecA2 also plays an important role in virulence in some bacteria [10–12]. Based on its interacting partner and substrates, it has been proposed that there are two types of SecA2 proteins. The first type is suggested to associate with the canonical Sec system and transports multiple type of substrates, and also called the SecA2-only system [13–15]. The second type is suggested to interact with the

accessory SecY2 channel, also called the SecA2/SecY2 system, and transport a single specific substrate completely independent from the canonical Sec system, [13]. Different types of SecA2-dependent proteins have been identified, and these appear to be involved in different functions and cellular locations. Some of these substrates are cell envelope proteins while others are secreted. Intriguingly, some substrates possess a signal sequence while others do not. However, the mechanisms by which SecA2 selects the substrates for transport is essentially unknown, nor is it clear why the substrates are not recognized by the canonical SecA. One hypothesis is that the absence of the HWD in SecA2 might result in a more solvent-exposed signal peptide binding cleft that could help SecA2 in the recognition of specific SecA2 substrates, including signal peptide-less variants [92].

2.2. SecY2, the accessory membrane channel

In addition to SecA2, some Gram-positive bacteria, e.g., *Streptococcus*, also possess the accessory SecY2, that is homologous to SecY [8,13]. SecY2 is predicted to form an accessory membrane channel that is responsible for the export of specific proteins, which cannot be exported by the canonical SecYEG. In general, SecY2 proteins share a low sequence similarity to SecY (SecY1). However, the predicted membrane topology of SecY2 is identical to that of SecY [13]. The conserved residues in cytoplasmic loop 5 of SecY, that are important for SecA interaction [95] are absent in SecY2, possibly suggesting a lack of interaction with the canonical SecA1 protein. In *Streptococcus* and *Staphylococcus*, SecY2 works together with SecA2 to export large serine-rich repeat (SRR) glycoproteins to the cell surface [8,96]. Therefore, it is suggested that SecY2 directly associates with SecA2 to form the functional Sec translocase. SecY2 is also suggested to associate with some potential accessory secretion proteins (Asps), i.e., Asp1-Asp5 [13]. Asp4 and Asp5 have been suggested to form the membrane channel with SecY2, and thus function similar to SecE and SecG, respectively. However, this Asps are absent in other bacteria that do contain a SecY2, and thus the exact composition of this accessory Sec-translocase has remained elusive.

3. The accessory Sec system in different type of Gram-positive bacteria

Gram-positive bacteria have a less complex cell envelope structure as compared to Gram-negative bacteria. Most of Gram-positive bacteria comprise only a single cytoplasmic membrane followed by a cell wall and defined as monoderm species. However, some Gram-positive bacteria possess an extra membrane, a peptidoglycan-mycolic acid wall structure, thus are diderm species, e.g., Mycobacteria. These are included as well in the discussion in this paragraph. The

accessory Sec system has been identified in almost three dozen Gram-positive bacterial species. Some of them have been well-characterized and there are some interesting similarities and differences between them. In most cases, the presence of accessory Sec components is closely associated with functions in intracellular survival and virulence. Below, we will discuss in detail of the accessory Sec system in different Gram-positive bacterial species. We will divide the bacterial species into two groups based on their accessory Sec type: SecA2 only systems and SecA2/SecY2 systems.

3.1. Bacterial species with SecA2-only system

3.1.1. *Mycobacterium* species

Mycobacteria possess the Sec transport systems for the translocation of proteins across the cytoplasmic membrane. All essential Sec components are present in Mycobacteria, both in pathogenic species, and non-pathogenic species. Interestingly, all Mycobacteria also possess the accessory SecA2, but they lack a SecY2, and thus the system is called the SecA2-only system [7,11]. Mycobacterial SecA2 proteins share only about 50% similarity with their corresponding SecA homolog (SecA1). Structural studies in *M. tuberculosis* show that SecA2 is smaller in size, as compared to SecA1, due to some deletions in C domain as discussed earlier. Functional studies in *M. tuberculosis* and *M. smegmatis* show that SecA1 is essential and likely it functions as the housekeeping SecA similar to *E. coli* SecA [7]. In contrast, SecA2 is non-essential since the deletion mutants could be constructed in several Mycobacteria, including *M. tuberculosis* [97], *M. smegmatis* [7], and *M. marinum* [98]. Cell fractionation studies in *M. smegmatis* show that SecA2 is predominantly cytosolic, while SecA1 is equally distributed between membrane and cytosolic fractions [14]. SecA1 and SecA2 have independent functions in protein export [7] and are present in equivalent amounts in *M. tuberculosis.* The role of SecA2 appears limited to only a subset of proteins. Similar to SecA1, SecA2 also bears ATPase activity which is required for SecA2-mediated protein export in both *M. tuberculosis* [99] and *M. smegmatis* [14]. SecA2 is also important for the virulence of *M. tuberculosis* [97,100], and *M. marinum* [98].

Mycobacterial SecA2 appears to transport different types of substrates. Proteomic studies in the non-pathogenic *M. smegmatis* identified Msmeg1704 and Msmeg1712 as SecA2-dependent proteins [101]. Both are sugar-binding proteins and contain a predicted N-terminal lipoprotein sequence. In *M. tuberculosis*, two SecA2-dependent proteins were identified: superoxide dismutase A (SodA) and catalase-peroxidase (KatG) [97]. These two proteins play a role in surviving

oxidative stress [97]. Interestingly, both proteins lack a signal sequence. A proteomic study in *M. marinum* revealed protein kinase G (PknG) as a SecA2-dependent protein which is important for virulence [102]. Similar to SodA and KatG in *M. tuberculosis*, PknG also does not possess a signal sequence. Although these proteins lacking a signal sequence can be translocated via the Sec pathway [103], it is unclear how SecA2 specifically recognize these substrates and how they are targeted to the Sec pathway. Recent studies in *M. smegmatis* suggest that protein export by the Mycobacterial SecA2 is determined by the preprotein mature domain instead of a signal sequence [104]. Interestingly, the study also showed that the mature domain of SecA2 substrates can also be exported by the Twin-arginine translocation (Tat) pathway when fused to a signal peptide for the Tat pathway. This suggest that SecA2 substrates may have a tendency to fold prior to export [104], and that SecA2 facilitates the targeting and export of such unique substrates.

In Mycobacteria and other organisms with SecA2-only system, SecA2 is suggested to work together with the canonical SecYEG protein. A SecA2-SecYEG association has been proposed based on a genetic study in *M. smegmatis* [105]. Additionally, structural conservation of SecA-SecY contact sites in SecA2 structure are in line with the expected interaction between SecA2 and SecYEG [92]. However, there is no evidence that SecA2 indeed directly interacts with SecYEG. SecA1 depletion studies in *M. smegmatis* indicate that SecA2-dependent proteins also depend on SecA1 [14]. Recent *in vitro* studies shows that the *M. tuberculosis* SecA2 interacts with itself to form a homodimer, but that it can also interact with SecA1 to form a heterodimer [106]. Possibly, SecA2 interacts with the SecA1/YEG translocase via an interaction with SecA1, explaining why SecA2 substrates also require SecA1 for translocation while SecA2 based on cell fractionation studies is mostly cytosolic.

3.1.2. *Listeria* species

Listeria species possess SecA2 but lack SecY2, similar to Mycobacteria [9]. Sequence alignments predict that SecA2 of Listeria harbors all functional domains found in SecA1, including HWD [13]. *L. monocytogenes* SecA2 is not essential for growth, but involved in virulence and protective immunity [9,10]. SecA2 is needed for the export of a large number of proteins, and by proteomic studies at least 17 substrates were identified, some of which carry a signal sequences, while others do not [10,107,108]. Two of SecA2-dependent proteins that contain a signal sequence have been studied in more detail. These are p60 (protein of 60 kDa) also called CwhA (Cell wall hydrolase A), and NamA also called MurA (N-acetylmuramidase A) [9,10,109]. SecA2-dependent proteins without a signal sequence include a homolog of SodA, MnSOD (manganese superoxide dismutase) [10,107], and LAP

(Listeria adhesion protein) [110]. All substrates studied thus far appear to function in virulence [10,109,110].

Recent studies in *L. monocytogenes* revealed that SecA2-dependent protein secretion requires SecA1 [111]. This data supports the hypothesis mentioned earlier that in bacteria with SecA2/only system, SecA2 works together with the canonical SecYEG/SecA1 system. Another study shows that the polar-localized cell division protein DivIVA is required for the translocation of the SecA2-dependent proteins p60 and NamA, and it was suggested that DivIVA influences the activity of SecA2 [112]. *SecA2* was also identified in non-pathogenic species such as *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. grayi* and *L. marthii*, but the exact role of SecA2 in those species is unknown. In *L. innocua*, SecA2 is involved in the translocation of NamA [113]. Another study in a non-pathogenic listeria on LAP translocation failed to provide evidence that SecA2 promotes bacterial adhesion. However, the lack of an effect might be due to the low level of expression of LAP in this organism [114].

3.1.3. *Clostridium difficile*

Clostridium difficile is the only clostridial species found to possess the SecA2 protein. *C. difficile* SecA2 is mostly cytosolic, whereas SecA1 localizes to the membrane [115]. In contrast to most of bacterial SecA2 which are not essential, SecA2 of *C. difficile* is essential for viability [115]. Additionally, the *C. difficile* SecA2 shows high homology to the SecA1 protein (~80% similarity). The *C. difficile secA2* gene is encoded within a gene cluster encoding surface layer (S-layer) proteins. These proteins form a paracrystalline sheets that assembles at the cell surface. Two proteins have been identified as the major substrates of the *C. difficile* SecA2 system, the S-layer protein (SlpA), which is the main component of the S-layer, and a cell wall protein (CwpV) [115]. SlpA and CwpV both contain a signal sequence. SlpA is essential for viability, suggesting the essential function of SecA2 in *C. difficle.*

3.1.4. *Bacillus* species

The accessory SecA2 protein is present in some Bacillus species such as *Bacillus anthracis. B. cereus, B. thuringiensis, B. smithi,* and *B. methanolicus*, but absent in *B. subtilis.* In *B. anthracis,* SecA2 is encoded within an S-layer gene cluster, similar to the *C. difficle* SecA2. However, the *B. anthracis* SecA2 is not essential for viability, but important for the translocation of a surface array protein (Sap) and the extractable antigen 1 (EA1), which are two major components of the *Bacillus* S-layer [116]. Sap is encoded in the *secA2* locus, whereas EA1 is encoded in other

genomic region. Apparently, SecA2 also is specialized for the translocation of S-layer protein in multiple organisms. *B. anthracis* also possess the accessory SecY2 protein, but this protein appears not essential for the translocation of the aforementioned SecA2 substrates, thus it belongs to the group of SecA2-only system. *B. anthracis* SecA2 associate with the accessory secretion factors, SlaP and SlaQ [116,117]. This two proteins are encoded immediately downstream of the *secA2* gene. Thus it appears that SecA2 functions together with SlaP and SlaQ to promote the S-layer assembly in *B. anthracis* [116,117]. SlaP and SlaQ are also present in other pathogenic bacillus species, such as *B. cereus*, and likely fulfil a similar function.

3.2. Bacterial species with SecA2/SecY2 system

3.2.1. *Streptococcus* species

Streptococcus species possess both the SecA2 and SecY2 protein that form the SecA2/SecY2 system. These proteins were originally identified and characterized in *Streptococcus gordonii* [8], and *S. parasanguinis* [118]. The predicted domain organization of Streptococcal SecA2 is similar to SecA2 of other organisms that lack SecY2. However, the Streptococcal SecA2 functions independent of the canonical Sec system, and likely forms a separate Sec system with SecY2 [8]. Cell fractionation studies in *S. parasanguinis* show that SecA2 is mostly associated with the membrane [119], in contrast to other bacterial SecA2s which are predominantly cytosolic. SecA2 and SecY2 are not essential for viability or the translocation of most secretory proteins [13]. In contrast to the multiple type of substrates in the SecA2-only system, Streptococcal SecA2/SecY2 system exports more specific substrate. The system appear to be specialized in the transport of serine-rich repeat (SRR) glycoproteins [8,13]. SRR glycoproteins are a family of adhesins in Gram-positive bacteria that fulfil an important role in pathogenicity [120,121].

In *S. gordonii,* SecA2 and SecY2 play an important role in the translocation of GspB. GspB is a serine-rich glycoprotein that mediates the binding of *S. gordonii* to platelets, and is encoded within the *secA2/secY2* gene cluster. GspB contains a relatively long signal sequence (90 amino acids) and a specific domain called the accessory Sec transport (AST) domain, that is essential for targeting to the SecA2/SecY2 translocase [122]. The *S. gordonii* SecA2/SecY2 system also includes other accessory secretion proteins (Asps), that are all encoded within the same operon; Asp1 to Asp5. Asp4 and Asp5 show homology to the canonical SecE and SecG proteins, respectively [123]. They are predicted to form the translocation channel together with SecY2 but this remains to be demonstrated. Asp1, Asp2, and

Asp3 interact with each other and are required for the translocation of GspB [124]. Asp2 and Asp3 directly interact with GspB via the SRR domain [125]. Both proteins also interact with SecA2, which may also be involved in the targeting of GspB preprotein to the translocation site [124,126,127]. Asp2 is important for the correct glycosylation of GspB during translocation [128]. Several cytosolic glycocylation factors, such as GftA (Gtf1), GftB (Gtf2), Gly and Nss, are involved in the glycosylation of GspB prior to translocation [121,129,130]. Therefore, one of the key features of this system is that the non-canonical Sec translocase translocated glycosylated precursor proteins.

In *S. parasanguinis,* SecA2/SecY2 are important for the translocation of Fap1, a homolog of GspB [118]. *S. parasanguinis* possess also homologs of Asp1, Asp2 and Asp3, termed glycosylation-associated protein 1 (Gap1), Gap2 and Gap3, respectively, but seems to lack the Asp4 and Asp5 proteins. Similar to *S. gordonii* Asps, *S. parasanguinis* Gaps are also encoded in the same operon as *secY2, secA2,* and also *fap1*. Gap1 and Gap3 interact with SecA2 *in vitro* [131], and both proteins are suggested to be involved in the complete glycosylation of Fap1 [121]. Based on the combined studies in *S. gordonii* and *S. parasanguinis*, it is suggested that Gap1-3 (Asp1-3) form a single functional complex with dual functions: first, it is involved in targeting the partially glycosylated substrate to the SecA2/SecY2 translocase, and second is needed for the complete glycosylation of the substrate during translocation [13,121]. *In vitro* study in *S. parasanguinis* shows that SecA2 and Gap3 associate with the canonical SecA1 [131], suggesting cross talk between the accessory and the canonical Sec system. However, the biological function of this association has not been established. In general, preproteins that are exported by the Streptococal accessory Sec system are glycosylated and cannot be exported by the canonical Sec system [13]. However, glycosylation is not required for protein translocation in the accessory Sec system, since the unglycosylated substrate can still be secreted by this system [132]. Interestingly, the non-glycosylated versions of GspB and Fap1 can also be translocated by the canonical Sec system [132,133], suggesting that glycosylation may take part in the targeting of these substrates to the accessory Sec system.

3.2.2. *Staphylococcus* species

The Staphylococcal accessory Sec system consists of both SecA2 and SecY2, similar to Streptococcus. The two proteins were discovered in *Staphylococcus aureus* [96] and found to be present also in some of other staphylococcal species; *S. epidermidis, S. warneri,* and *S. carnosus.* In *S. aureus,* SecA2 and SecY2 are not essential for viability, but required for translocation of SraP (serine-rich adhesin for

platelets) [96]. However, it is still need to be verified whether SraP is selectively translocated via the SecA2/SecY2 system or not. SraP is a homolog of GspB of *S. gordonii*, and fulfils a role in binding to human platelets [134]. The accessory Sec locus of *S. aureus* is similar to that of *S. gordonii*. It also contains the genes encode Asp1-Asp3 that all are required for the transport of SraP [135]. However, *S. aureus* lacks four proteins present in *S. gordonii*, i.e., Gly, Nss, Asp4 and Asp5. Gly and Nss are important for the glycosylation of GspB in *S. gordonii*, suggesting that there is a difference in the glycosylation mechanism between staphylococcus and streptococcus. Asp4 and Asp5 are predicted to be the part of accessory membrane channel together with SecY2 in *S. gordonii*. The absence of Asp4 and Asp5 in *S. aureus* indicates that the requirements for these Asps is specific for some species, i.e., *S. gordonii,* while other proteins might function as partner subunits of the *S. aureus* SecY2. Interestingly, genetic study suggests that *S. aureus* SecY2 function together with SecG [135]. Since *S. aureus* lacks a second set of *secE* and *secG* genes, these findings suggest that the *S. aureus* SecY2 might form an alternative translocation channel with SecE1 and SecG1.

3.3. Other bacteria

The accessory Sec system is more wide-spread in Gram-positive bacteria, but mostly poorly characterized. Among these is *Corynebacterium glutamicum* that possess the SecA2-only system. *C. glutamicum* SecA2 is essential for viability [136], but no specific substrates have been identified thus far. The accessory SecA2 and/or SecY2 proteins are also present in some of *Gordonia*, *Pediococcus*, *Enterococcus*, and *Lactobacillus* species [13], but not further characterized.

4. Conclusions

The canonical Sec translocase is omnipresent in bacteria, and similar systems exist in Gram-negative and positive bacteria. In contrast, accessory Sec components are found only in Gram-positive bacteria where they fulfil diverse functions in protein secretion. During the last fifteen years, a better understanding on Gram-positive bacterial accessory sec system has been obtained. Since this system also plays an important role in bacterial virulence, the studies also revealed mechanisms of pathogenesis and potentially, the system may function as a possible drug target. The current model of the accessory Sec system is shown in Fig. 4, which is based on the combined studies discussed above.

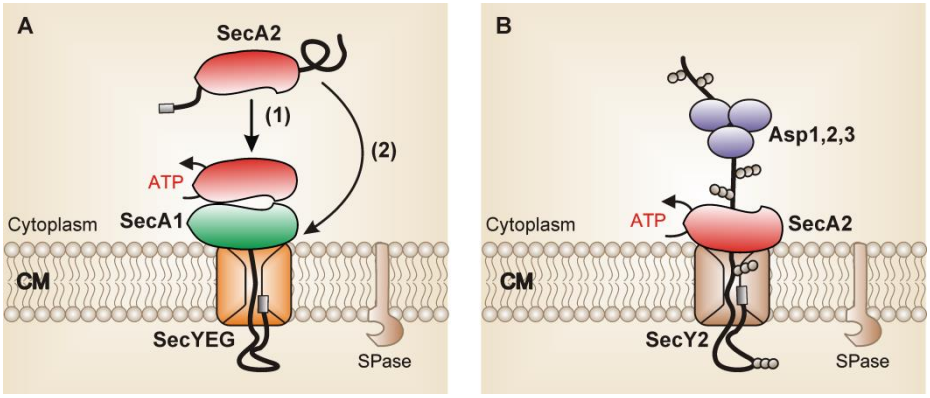

Figure 4. The accessory Sec system. (A) SecA2-only system. SecA2-dependent proteins are recognized by the accessory SecA2 (red) and are targeted to the SecYEG channel (orange), either via (1) the housekeeping SecA1 (green) or (2) direct interaction of SecA2 with SecYEG. The ATPase activity of either SecA2 or SecA1 or both provides the energy for the translocation of the proteins through the SecYEG pore. (B) SecA2/SecY2 system. Partially glycosylated preproteins are targeted to the accessory SecA2 protein (red) by the Asp1-3 (Gap1-3) complex (purple). During the translocation process, the Asp1-3 (Gap1-3) complex modifies the glycan composition and completes the glycosylation of the preproteins (brown dot). The ATPase activity of SecA2 provides the energy for the translocation of the fully glycosylated preprotein through the SecY2 membrane channel (brown). SPase: signal peptidase; CM: cytoplasmic membrane.

5. References

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